PART I. BIOLOGY OF α-FETOPROTEIN

# NORMAL BIOLOGY OF α-FETOPROTEIN

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Synthesis of α-fetoprotein (AFP) by the human embryo can be detected at least as early as 29 days after conception.[1] At this stage of development, the liver is little more than a diverticulum of hepatic buds and ducts, and there is only slight, if any, evidence of lobe formation. Yet, this embryonic liver can synthesize not only AFP, but a host of other plasma proteins as well, including prealbumin, albumin, $\alpha_1$-antitrypsin, C1-esterase inhibitor, $\alpha_2$-macroglobulin, C3 or $\beta_{1C}$, β-lipoprotein, hemopexin and transferrin.[2] A few days later, at 32 to 35 days after conception, the lobes of the liver can be discerned, and hepatic synthesis of $\alpha_1$-acid glycoprotein and ceruloplasmin becomes apparent.[2] By 38 to 40 days of gestation, an occasional embryo may demonstrate hepatic synthesis of fibrinogen, but most embryos do not synthesize fibrinogen until about 60 days of gestation.[2] Thus, early morphologic differentiation of the human liver is accompanied by the development of hepatic plasma protein synthesis.

Hepatic synthesis of AFP takes place in the hepatocyte.[3] The liver, however, is not the only source of AFP in the normal conceptus. At 32 to 35 days of gestation, the amount of AFP produced by the yolk sac rivals that synthesized by the liver.[1, 4] At this stage of development, the yolk sac is a well-defined vesicle attached to the foregut by a narrow stalk, and it produces prealbumin, albumin, $\alpha_1$-antitrypsin and transferrin, among other proteins, in addition to AFP (FIGURE 1); with the exception of albumin, these proteins are synthesized in quantities equal to or even greater than those produced by equivalent amounts of liver from the same embryo. Synthesis of AFP, prealbumin, $\alpha_1$-antitrypsin and transferrin by the yolk sac is significantly decreased at 60 days or 8.5 weeks of gestation (TABLE 1). By 11.5 weeks of gestation, the yolk sac may be noticeably atretic; its synthesis of prealbumin and $\alpha_1$-antitrypsin may be below detectable limits, and it produces only small amounts of AFP, albumin and transferrin. Thus, during its relatively brief existence as a well-developed structure, the yolk sac of the human conceptus synthesizes a number of plasma proteins, but as degenerative changes occur towards the end of the first trimester or the beginning of the second, the changes are accompanied by a decrease in plasma protein synthesis. In contrast to the relative insignificance of the yolk sac in human development, the yolk sac in the rat fetus and the yolk sac in the chick embryo remain well-developed structures with biologically important functions throughout antenatal development: in the rat, the yolk sac forms one of the fetal membranes and in the chick, the yolk sac envelops and absorbs the yolk necessary to nourish the embryo. The yolk sac in both of these species produces AFP until birth.[5, 6]

Synthesis of AFP in the human conceptus also takes place in the gastrointestinal tract,[1] but the quantity produced is very much less than that synthesized by the liver. Trace amounts of AFP may also be produced by the kidneys in an occasional conceptus, or even by the placenta, but synthesis at either site

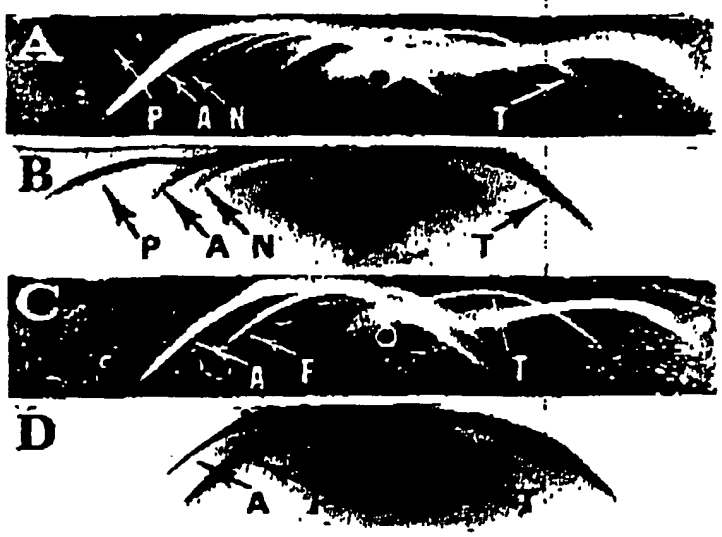


FIGURE 1. Immunoelectrophoresis (A) and corresponding autoradiograph (B) of yolk sac culture, embryo of 5.5 weeks of gestation, with adult serum added as carrier and developed with rabbit antiserum against adult serum proteins. Immunoelectrophoresis (C) and autoradiograph (D) of same yolk sac culture as in A, but with fetal serum added as carrier and developed with rabbit antiserum against fetal serum proteins. Arrows P, A, N, F, and T indicate prealbumin, albumin, $\alpha_1$-antitrypsin, AFP and transferrin, respectively. (From Gitlin and Perricelli.[4] By permission of the publishers of *Nature*.)

does not seem to be common,[1, 7] and the quantities produced are very small indeed.

Early in human development, then, the liver and the yolk sac are the principal sources of the AFP present in embryonic plasma. Although the quantity of AFP produced by the yolk sac equals that produced by an equivalent weight of liver during the middle third of embryonic development, i.e., from 4 to 8 weeks, the liver soon surpasses the yolk sac in size, and becomes the greater source of AFP. The concentration of AFP in the plasma of the embryo at 45 days or 6.5 weeks of gestation is approximately 67 $\mu$g per ml and rising rapidly.[8] In the next 3 weeks, i.e., by 9.5 weeks of gestation, the AFP level increases some

TABLE 1

PRESENCE OF RADIOACTIVE SERUM PROTEINS IN YOLK SAC CULTURES AS DETERMINED BY RADIOIMMUNOELECTROPHORESIS

| Gestational Age (Weeks) | Prealbumin | Albumin | α-Feto-protein | $\alpha_1$-Anti-trypsin | Transferrin |
|---|---|---|---|---|---|
| 5.5 | ++++ * | ++ | ++++ | ++ | ++++ |
| 5.5 | ++++ | ++ | ++++ | ++ | ++++ |
| 8.5 | + | ++ | +++ | + | ++ |
| 11.5 | 0 | + | + | 0 | + |

* 0=not detected; + to ++++=increasing degrees of intensity of precipitation line on autoradiograph. (From Gitlin and Perricelli.[4])

30-fold to almost 2000 μg per ml (FIGURE 2). By this time, the liver is much larger than the yolk sac, and the synthesis of AFP by the yolk sac has decreased absolutely as well as relatively. Thus, the increase in the plasma AFP level between 6.5 and 9.5 weeks of gestation seems to be attributable to an increase in the rate of AFP synthesis by the liver. Somewhere between 10 and 13 weeks of gestation, the plasma concentration of AFP reaches a maximum that averages approximately 3000 μg per ml,[8] and virtually all of the AFP present in the conceptus at this point is of hepatic origin.

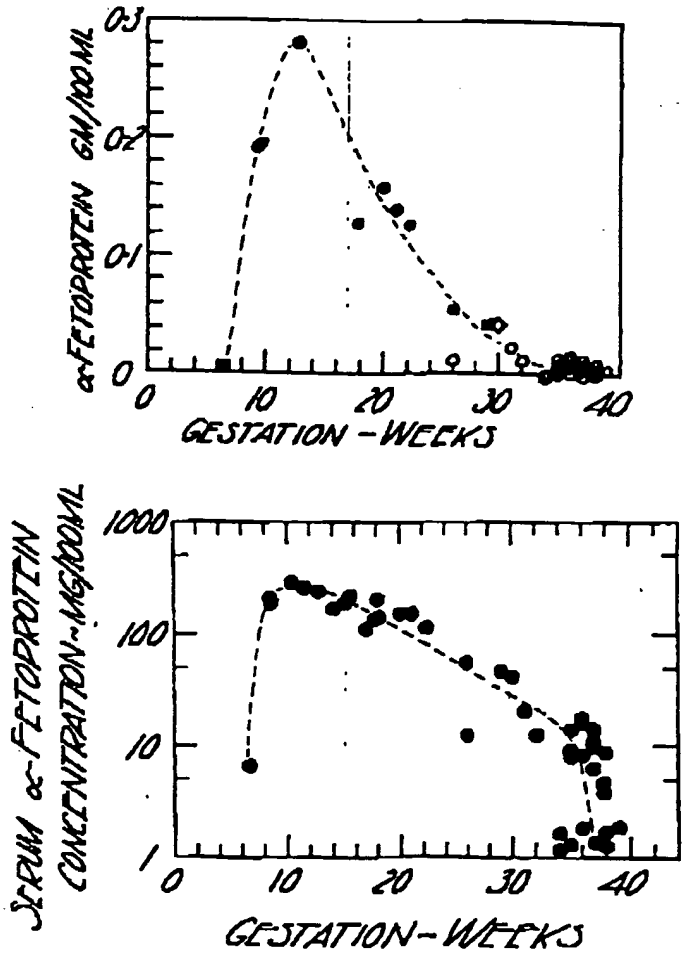


FIGURE 2. Concentration of AFP in the plasma of the human conceptus. *Top:* concentrations are plotted linearly and each point represents a single conceptus. Symbol indicates the manner of delivery: solid circles=hysterotokotomy; solid squares=incompetent cervix; hollow crossed circle=extraction for abruption of placenta; hollow circles=spontaneous. (From Gitlin and Boesman.[8] By permission of the publishers of *The Journal of Clinical Investigation.*) *Bottom:* concentrations are plotted semilogarithmically. Includes all infants in top figure plus additional infants studied later.

The concentration of any protein in the plasma is a balance between the rate at which the protein is synthesized, the volume of the body fluids into which the protein is distributed and the rate at which the protein is removed from the body whether by degradation or loss or both. Plasma proteins, of course, are not restricted to the vascular compartment of the body, and those that have a molecular weight similar to that of AFP are normally distributed almost equally between plasma and interstitial fluids.[8] Since the conceptus grows rapidly in the first trimester,[10] the embryo being but 20 mg at 4 weeks, 1 g at 8 weeks and 14 g at 12 weeks, both the vascular compartment and the interstitial fluid compartment are also increasing concomitantly. It should be clear, then, that while

the plasma AFP concentration rises quickly during this period, the total amount of AFP synthesized by the embryo increases even faster than the plasma concentration alone would indicate.

From about 14 weeks of gestation until approximately 30 to 32 weeks of gestation, the plasma concentration of AFP in the fetus declines.[8] This decline appears to be exponential (FIGURE 2) and is attributable to a combination of factors. If the plasma concentration of AFP at a given stage of development is multiplied by the weight of the fetus at that time, an approximation of the relative quantities of AFP synthesized by the fetus at different points in gestation may be obtained (FIGURE 3). The data reveal that the total quantity of AFP being synthesized by the fetus after 14 weeks of gestation increases even more rapidly than in the first trimester, despite the fact that the plasma concentration is falling at this time, and that this rate of increase is sustained until approximately 20 weeks of gestation. After 20 weeks of gestation, the total quantity of AFP synthesized remains relatively constant until 30 to 32 weeks of gestation. However, it should be noted that whereas the fetus weighs approximately 50 g at 14 weeks of gestation, the fetus at 20 weeks of gestation weighs 310 g, an increase in weight, and hence an increase in the volume of plasma and interstitial fluids as well, of more than 6-fold.[10] Yet, the total amount of AFP produced at 20 weeks is less than 5 times that produced at 14 weeks.[8] It will also be noted that during the period from 20 weeks of gestation to 32 weeks of gestation, the weight of the fetus increases 5.4 times, but total AFP synthesis remains constant. Thus, the decline in the plasma AFP concentration in the fetus from approximately 14 weeks of gestation until 32 weeks of gestation is due to the fact that the increase in the weight of the fetus during this period is greater than the increase in total AFP synthesis. Stated somewhat differently, the exponential fall in the fetal plasma level of AFP is attributable to an exponential decline in the amount of AFP synthesized per unit weight of conceptus. The rate at which the plasma level, and AFP synthesis, decreases has a half-life of 32 days.

At 32 weeks of gestation, the plasma AFP concentration in the conceptus is approximately 200 $\mu$g per ml. After about 32 to 34 weeks of gestation, the AFP level decreases precipitously to reach levels from 13 to 86 $\mu$g per ml at term (FIGURE 2). This rapid decline is apparently due to a relatively abrupt further

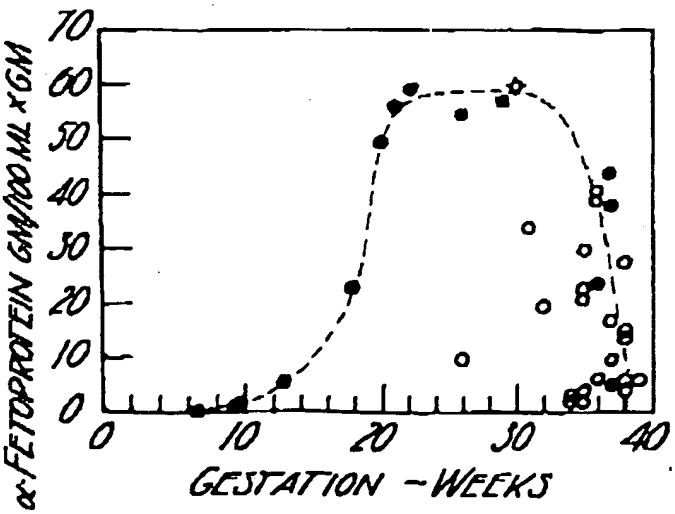


FIGURE 3. Plasma concentration of AFP multiplied by body weight of the conceptus. Symbols as in FIGURE 2. (From Gitlin and Boesman.[8] By permission of the publishers of *The Journal of Clinical Investigation*.)

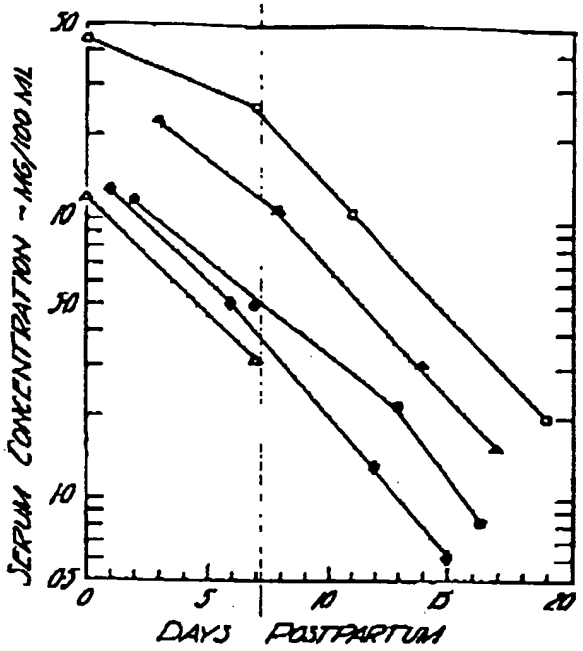


FIGURE 4. Disappearance of AFP from plasma in five infants during the immediate postnatal period. *Hollow triangles* indicate an infant of spontaneous delivery at 26 weeks of gestation; *hollow circles*, infant's delivery caused by incompetent cervix at 29 weeks of gestation; *solid triangles*, hysterotokotomy at 30 weeks; *solid circles*, spontaneous delivery at 36 weeks; and *solid diamonds*, spontaneous delivery at 36 weeks. (From Gitlin and Boesman.[8] By permission of the publishers of *The Journal of Clinical Investigation*.)

reduction in the rate of AFP synthesis. The wide range of plasma AFP levels encountered at delivery, and the differences that 1 or 2 weeks in gestation may make in the levels observed, suggest that caution should be used in taking the plasma AFP level at delivery as a definitive measure of either maturity or disease.

The plasma AFP level in the newborn normally declines rapidly with an average half-life of 3.5 days during the first weeks of life (FIGURE 4) and then somewhat more slowly until normal adult levels of 1 to 2 ng per ml are reached by 2 years of age.[11] The rate at which the level falls in the newborn period is compatible with the rate at which AFP is catabolized, suggesting that the synthesis of AFP at or near birth is almost completely repressed. In some infants, the half-life of AFP during the first days of postnatal life is somewhat greater than 3.5 days, indicating a brief delay in the curtailment of synthesis in these infants. In any event, synthesis evidently does not cease entirely at or near term, since very low concentrations of AFP are present in the adult.[12, 13] It should be noted, however, that the average plasma concentration of AFP found at birth is approximately 20,000 times that in the normal adult.

Serum AFP levels up to 500 ng per ml have been reported in normal pregnant women.[14] Interestingly, the average serum level of AFP appears to be highest during the third trimester, and usually is not elevated above the normal nonpregnant adult level during the first trimester.[15] As noted earlier, the maximum AFP level in the fetus is reached at the end of the first trimester or at the beginning of the second.[3] As the level in the mother rises from an average of 45 ng per ml at 16 weeks of gestation to 450 ng per ml at 32 weeks of gestation, the fetal concentration decreases from approximately $2 \times 10^6$ ng per ml to $2 \times 10^5$ ng per ml during the same period. Despite the decline in fetal plasma level, however, there is an obvious concentration gradient from fetus to mother throughout this period. Proteins such as albumin and transferrin pass between mother and fetus by a mechanism that is most probably diffusion:[16] the rate of transfer is first order and inversely proportional to the square

root of the molecular weight of the protein. Since the molecular weight of AFP is similar to that of albumin, it is not unreasonable to presume that the elevation of the AFP level in the mother may be due, at least in part, to transfer of the protein from the fetus to the mother by diffusion. Fetomaternal transfer of AFP, however, must be quite small even in the third trimester of pregnancy; at this point, the maximum amount of AFP transferred from fetus to mother can be estimated using the relationship $D = 0.693\ CV/t\frac{1}{2}$, where D is the rate at which AFP is degraded, C is the plasma AFP concentration, V is the volume of distribution for AFP and $t\frac{1}{2}$ is the half-life of AFP. It should be noted that V is not an anatomic parameter; V is defined as that volume which the total body pool of a protein would occupy, if the concentration of the protein in all body fluids were uniform and equal to the concentration in plasma.[9] For most proteins, $V = 0.1\ W$, where W is the body weight. Since the maternal plasma concentration of AFP at 32 weeks of gestation averages 450 ng per ml and the half-life of AFP is about 3.5 days, the average amount of AFP degraded at this time by a mother weighing 50 kg would be approximately 450 $\mu$g. At this point of maximum maternal AFP concentration, the maternal AFP concentration is neither rising nor falling, and the rate at which AFP enters the maternal AFP pool is equal to the rate at which it leaves the pool, or in this instance, 450 $\mu$g per day. The rate of 450 $\mu$g per day would represent the maximum transfer of AFP from fetus to mother at 32 weeks, assuming that the mother synthesizes no AFP. If the mother synthesizes AFP at a rate equal to that in the normal adult the maternal contribution would be less than 2 ng per day. At a plasma concentration of 200 ng per ml in an average 32-week fetus weighing 1670 g, fetomaternal transfer of 450 $\mu$g per day would require the clearance of 1.75 ml of plasma, or the amount of AFP present in less than 1.1% of the fetal body pool.

If 450 $\mu$g of AFP per day were infused into a 50 kg woman having an initial plasma concentration of 1 or 2 ng per ml, the plasma concentration would be close to 450 ng per ml within 3 weeks. Yet, plasma AFP concentrations in the pregnant woman do not reach this level until approximately 32 weeks of gestation. Obviously then, either the amount transferred from fetus to mother is much less than 450 ng per day before the third trimester, or much of the AFP in the mother is of maternal rather than fetal origin. The amount of AFP that can be transferred from fetus to mother will depend upon: (1) the permeability of the fetomaternal barrier, which in this instance is primarily the placenta; (2) the area of the permeable barrier, or for practical purposes, the size of the placenta; and (3) the fetal AFP concentration. The placenta weighs approximately 150 g at 20 weeks of gestation and 500 g at 32 weeks,[17] an increase of 3.3-fold; on the other hand, the maternal plasma AFP concentration increases 9-fold in the same period. In addition, the fetal plasma concentration of AFP at 32 weeks of gestation is only 15% or less of that at 20 weeks, and hence *decreases* some 7-fold during this period. It is apparent, then, that if a significant fraction of the AFP present in the mother at 32 weeks comes from the fetus, there must be an increase in placental permeability to AFP during the second trimester. Whether the permeability of the human placenta to AFP does increase with gestation is not known, but both human and murine placentas normally become increasingly pervious to a number of plasma proteins during the last half of gestation.[16, 18]

It has been noted that some infants born as a consequence of the premature onset of spontaneous labor have plasma AFP levels lower than those found in

normal fetuses of comparable gestation.[5] It has also been observed that the maternal plasma level of AFP may be elevated following spontaneous abortion.[19] The data suggest the possibility that an increase in the permeability of the placenta to AFP may precede or accompany spontaneous abortion.

Plasma proteins may pass between mother and fetus not only via the placenta but also by way of amniotic fluid. Estimations of the average AFP concentration in amniotic fluid at 32 weeks of gestation differ, the range being from 425 ng to 15 μg per ml,[21, 22] but it is agreed that AFP is excreted by the fetus into the urine, which is then released into amniotic fluid.[5] The average amniotic fluid volume at 32 weeks is approximately 800 ml.[23] At a concentration of 15 μg per ml, the total amount of AFP in amniotic fluid would average 12 mg. Since approximately 0.23% of a given protein in amniotic fluid passes into the maternal body pool per day,[20] the maximum amount of AFP normally transferred from fetus to mother via amniotic fluid at 32 weeks of gestation would be, on the average, 27.6 μg, or only 6% of the amount necessary to sustain the maternal plasma concentration at 450 ng per ml. It is clear, however, that an increase in the amount of AFP in amniotic fluid for whatever reason could result in an increase in the amount of AFP entering the maternal pool, including maternal plasma, by this route.

Despite the fact that virtually all of the AFP present in amniotic fluid is derived from the conceptus, from 95 to 98% of all other serum proteins in amniotic fluid comes from the mother:[19] AFP accounts for less than 0.5% of the total soluble protein in amniotic fluid. A 2- or 3-fold increase in the serum proteins contributed to amniotic fluid by the fetus will double or triple the amniotic fluid AFP level, but the contribution will affect the total amniotic fluid protein concentration relatively little. Even a 10-fold increase in the fetal contribution to the serum proteins in amniotic fluid, such as might occur when the fetus has a serious open neural tube defect,[21] may not increase the total protein level in amniotic fluid much above normal, although the AFP level in amniotic fluid at the same time would be 10 times normal. A marked increase in the total protein level in amniotic fluid should suggest a serious compromise in fetal physiology, since more than 60% of all protein in amniotic fluid is cleared daily by fetal swallowing.[20]

As noted earlier, the normal adult plasma level of AFP is approximately 1 to 2 ng per ml, or 1/20,000th of the plasma level at birth.[12, 13] In the normal course of events, the developmental sequence of AFP synthesis in the male more or less terminates at 2 years of age unless certain hepatic, germ cell, or gastrointestinal afflictions supervene: at 2 years of age, the plasma AFP concentration reaches adult levels and these are maintained until death.[11] In the female, pregnancy imposes its own unique AFP cycle, and provides for the beginnings of a new cycle—the conceptus.

All mammalian species thus far studied, including subhuman primates, carnivores, rodents, lagamorphs, ruminants, edentates, and marsupials, synthesize one or more homologues of human plasma AFP during fetal development.[27–37] Studies of the antigenic structure of these AFPs using specific rabbit antisera have revealed that the AFPs of different mammals have both similarities and differences in their molecular structure. Some of the antigenic determinants present on human AFP, for example, are similar to some of the determinants found on the AFPs of not only the subhuman primates but also of such diverse species as the armadillo, the sheep, and such carnivores as the harbor seal, dog, and cat. On the other hand, human AFP is antigenically, and hence structurally,

different from the AFPs of other mammals including the subhuman primates; primate AFPs in turn have antigenic determinants that are different from those on the AFPs of carnivores, and the latter have determinants not detected on the AFPs of many other mammals such as the sheep, armadillo, rat, and mouse, among others.

Some mammals have 2 or more variants of AFP. In the rat, for example, AFP is represented by 2 molecular species readily separated by electrophoresis,[25, 28] but immunochemically indistinguishable from each other; each of the 2 AFPs demonstrate similar molecular microheterogeneity.[29] The cycle of AFP synthesis in the rat fetus is quite different from that in the human conceptus, due in part to sustained synthesis of AFP by the yolk sac throughout gestation.[5]

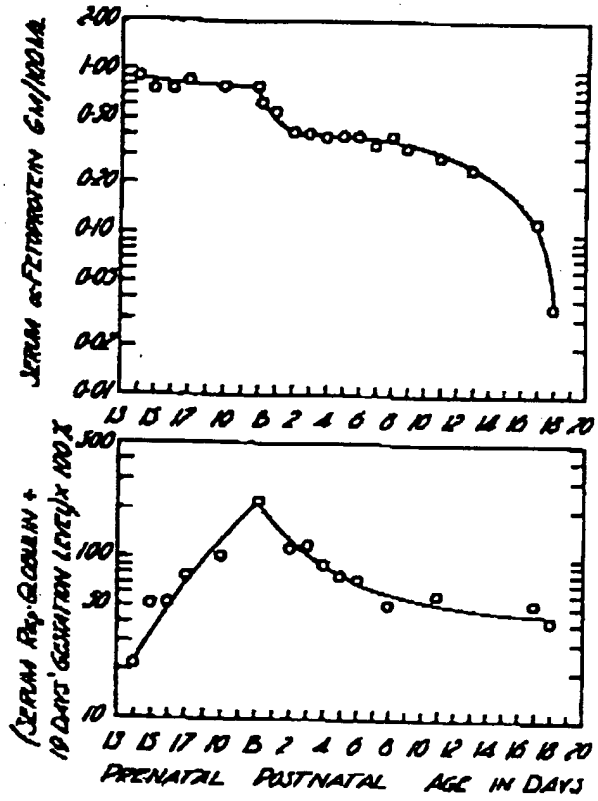


FIGURE 5. Plasma concentrations of AFP and Rα$_2$-globulin in the rat as a function of age. (From Gitlin and Boesman.[5] By permission of the publishers of *The Journal of Clinical Investigation.*)

From approximately mid-gestation to term, the plasma AFP concentration in the rat fetus is maintained at approximately 8 mg per ml; there is a slight decrease in concentration as term approaches (FIGURE 5), but this decline is relatively slight compared to the marked fall observed in the human fetus. The plasma AFP level in the neonatal rat falls rapidly after delivery until a level about half of that present at birth is reached. This rapid decline in AFP concentration is due to the loss of the yolk sac during parturition: as stated earlier, the yolk sac in the rat forms one of the fetal membranes, and the fetal membranes, of course, are discarded during delivery. The data suggest that, at term, the yolk sac synthesizes about half of the total AFP. The rate of decrease in the plasma AFP level after birth indicates that the half-life of AFP in the neonatal rat is approximately 1 day. After this initial postnatal fall, the plasma concentration of AFP

in the newborn rat is sustained for about a week at half the prenatal level, and then declines at an increasing rate of fall in the following weeks. This second postnatal decline is attributable to an increasing reduction in hepatic AFP synthesis during the second postnatal weeks. The adult rat, like the adult human, synthesizes relatively minute quantities of AFP.

A homologue of mammalian AFP, $\alpha_f$-globulin, is synthesized by birds.[4, 30] However, in birds, $\alpha_f$-globulin is produced principally by the yolk sac: if the protein is synthesized by the liver, the amounts produced are so small as to escape detection by the methods used for study.[4]

Sharks also synthesize a plasma α-globulin that seems to be a homologue of mammalian AFP.[31] Shark AFP, like human AFP, is synthesized primarily in embryonic or fetal tissues arising from the entoderm, viz., the liver, yolk sac, and gastrointestinal tract. Shark AFP has a molecular weight of 75,000 daltons: the molecular weight of human AFP is 65,000 to 70,000 daltons. Synthesis of shark AFPs, like mammalian AFP, begins early in the first trimester, and is sharply reduced at or near birth.

Since it is unlikely that AFP appeared in mammals, birds and sharks as independent evolutionary events, it would seem that an archaic homologue of AFP probably existed in species that were common ancestors to these classes. The evolutionary path that led to the development of the elasmobranchs diverged from that leading to the development of mammals at some time during the Silurian period of the Paleozoic era; an ancestral homologue of human AFP, therefore, probably existed at least 400 to 450 million years ago. There also seems to have been an orderly change in emphasis among the entodermally derived sites of AFP synthesis during evolution. Among sharks, the fetal stomach has a greater role in AFP production than does the gastrointestinal tract in mammals, but the shark yolk sac plays a smaller role than does the mammalian yolk sac. The liver in both sharks and mammals is a principal site of AFP synthesis, yet the avian liver has largely or completely lost the capacity to produce AFP; the primary site for AFP synthesis in birds is the yolk sac. During evolution, therefore, there was a shift in AFP synthesis towards the yolk sac and away from the stomach before or during the emergence of mammals and away from the liver at some point between the emergence of mammals and the development of birds.[31] Sic transit tempus!

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